REMARKS:

Claims 1, 7 to 10, 19 and 50 to 51 are pending under examination. Claims 1 to 7 are amended herein for clarity to more particularly define the invention. Support for these amendments is found throughout the specification and in the original claim language as set forth below. No new matter is added by these amendments and their entry is respectfully requested. The claims previously withdrawn from consideration by the Examiner have been cancelled herein without prejudice to the filing of a divisional application. In light of the amendments presented herein and the following remarks, applicants respectfully request reconsideration of the pending application and the allowance of the pending claims to issue.

I. REJECTION UNDER 35 USC § 101

The Examiner has rejected claims 1, 7 to 10, 19 and 50 to 51 under 35 USC § 101 on the grounds that the claimed invention lacks a specific, substantial asserted utility or a well established utility.

The Examiner argues that further experimentation is required to show that the claimed polynucleotides or the encoded polypeptides thereof have practical use.

It is respectfully submitted that the Examiner is incorrect in this characterization. Before the priority date of this application, April 27, 1998, the protein known as eps15 had been identified and was known to be a substrate of the EGF receptor tyrosine kinase. It was also known that over-expression of eps15 had a transforming effect on cells in vitro, indicating eps15 to be an oncogene - see, for example, the enclosed Abstract of Fazioli et al., (1993) Mol. Cell Biol., v. 9, pp. 5814 to 5828, enclosed.

Data had also been described suggesting that eps15 played a critical role in the recruitment of active EGF receptors into coated pit regions during endocytosis of ligand-occupied EGF receptors - see enclosed abstract of van Delft et al., (1997), J. Cell Biol., v. 137, pp. 811-821, enclosed. Studies on the internalization of EGF and

on endocytosis of the Sindbis virus had demonstrated that esp15 and esp15R are essential components of the endocytic machinery - Abstract of Carbone et al., (1997), Cancer Res., v. 57, pp. 5498 to 5504, enclosed.

It was therefore well known, before the priority date of this application, that esp15 was a target which could be employed to screen for potential pharmaceutical compounds affecting endocytosis. For example, it was known, before the priority date of this application, that endocytosis is involved in a number of disease states -- see, for example, enclosed Abstracts:

1. Magnifico et al., (Jan. 1998), "Heregulin beta 1 induces the down regulation and the ubiquitin-proteasome degradation pathway of p185 HER 2 oncoprotein", which discusses receptor endocytosis in the degradation of an oncoprotein;
2. Floyd et al., (Aug.1998), "Endocytosis proteins and cancer: a potential link?"; and
3. Krischer et al., (1993), "Endocytosis is inhibited in hepatocytes from diabetic rats."

There was therefore a real world need to screen for compounds affecting endocytosis, using any of the available targets.

As described in the subject application, the applicants have found that over-expression of Ese1 protein blocks endocytosis and Ese1 has also been shown to bind to eps15 (Specification, pages 18-19 and 22). This Ese1/eps15 interaction therefore provides a novel target for screening for potential pharmaceutical compounds to modulate the endocytic process by modulating the binding of Ese1 and eps15. Again, this is a real world application and a substantial utility of the present invention.

Such screening is discussed in the subject application, for example at page 6, lines 8 to 11.

Accordingly, it is respectfully submitted that the subject invention does have a specific, substantial asserted utility and a well established utility, in compliance with 35 USC 101. The Examiner is respectfully requested to withdraw this rejection.

II. REJECTION UNDER 35 USC §112, FIRST PARAGRAPH, ENABLEMENT

The Examiner has also rejected claims 1, 7 to 10, 19 and 50 to 51 as lacking enablement on the grounds that they are not supported by a specific, substantial asserted utility or a well established utility.

It is respectfully submitted that the arguments presented above with respect to utility indicate that the invention does have a specific, substantial asserted utility or a well established utility and therefore is fully enabled by the specification as filed. Withdrawal of this rejection is respectfully requested.

III. REJECTION UNDER 35 USC, § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

The Examiner has rejected claims 1, 9 to 10 and 19 under 35 USC 112, first paragraph, as lacking a clear written description. Claim 1 has been amended to delete reference to a nucleotide sequence at least 80% identical to SEQ ID NO: 1 or 2.

It is respectfully submitted that the specified nucleotide sequences of claim 1, ie. SEQ ID NO: 1 or 2 or a nucleotide sequence at least 90% identical to those sequences, represent a clearly defined genus of nucleotide molecules closely related in sequence to the nucleotide sequences of SEQ ID NO: 1 and 2.

Claim 1 has been amended to specifically recite a nucleotide sequence that is at least 90% identical to SEQ ID NO:1 or 2. It is not necessary to list all possible sequences having at least 90% sequence similarity to SEQ ID No1 or 2. Methods of determining sequence similarity and of obtaining degenerate sequences are well-known and readily available in the art. One skilled in the art would be able to readily

envision a genus of nucleotide sequences having the recited level of sequence similarity to SEQ ID NO:1 or 2. Thus, claim 1 as amended recites a genus of nucleotide sequences having close structural similarity, which may be readily envisioned by the skilled worker.

Further, the USPTO has specifically addressed the issue of written support for a genus of nucleic acid molecules having a high degree of structural similarity. To illustrate, Example 9 ("Hybridization") of the Revised Interim Written Description Training Examples addresses a situation similar to that raised in the instant Office Action. In Example 9, the exemplary claim recites an "isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO:1." The application used in Example 9 discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to the dopamine receptor and stimulates adenylate cyclase activity. In the "Analysis" section for this Example, it is stated:

> Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Likewise, Claim 1 recites molecules having a high degree of structural similarity. Accordingly, Applicants submit that the description in the specification in view of the high degree of structural similarity among the recited nucleic acid molecules and the high level of skill and knowledge in the art provides an adequate written description for nucleotide sequences having at least 90% identity to SEQ ID NO:1 or SEQ ID NO:2.

Thus, the pending claims are directed to a genus of molecules having close structural and functional similarity, such that one skilled in the art would recognize that Applicants were in possession of the claimed invention at the time of filing.

Further, the Examiner argues that these claims are rejected for the reasons already of record in the paper of 01/20/04. It is respectfully submitted that the reasons given in paper 01/20/04 were directed to the previously unamended claims of this application which were drawn, for example, to "an isolated nucleic acid comprising a nucleotide sequence encoding a "mammalian" or "murine" or "human" Ese1." It is respectfully submitted that the reasons given in the paper of 01/20/04 are not relevant to amended claim 1 now under examination.

The Examiner is respectfully requested to withdraw this rejection.

IV. REJECTION UNDER 35 USC, § 112, FIRST PARAGRAPH, SCOPE

The Examiner has rejected claims 1, 9 to 10 and 19 under 35 USC 112, first paragraph, on the grounds that the specification does not enable a nucleotide sequence that is 80% or 90% identical to SEQ ID NO: 1 or 2.

Claim 1 has been amended to remove reference to a sequence at least 80% identical to SEQ ID NO: 1 or 2, as noted above.

The Examiner argues that one of skill in the art would not know how to make the claimed polynucleotides such that they would function as claimed.

It is respectfully submitted that the Examiner is incorrect and that one of skill in the art would certainly be able to readily prepare the claimed polynucleotides and could readily determine whether or not such molecules have the described function. This would not involve undue experimentation but would merely require routine screening and application of routine technology.

It is respectfully submitted that the claimed nucleotide sequences are fully enabled by the specification as filed. Withdrawal of this rejection is respectfully requested.

V. REJECTION UNDER 35 USC § 112, FIRST PARAGRAPH, SCOPE

The Examiner has rejected claim 8 under 35 USC 112, first paragraph, in view of the language "SEQ ID NO: 1 or 2 or a complete complement thereof as a primer". It is believed that the Examiner's objection to claim 8 arises from the wording of claim 7, from which claim 8 depends. Claim 7 has therefore been further amended to clarify its meaning. It is respectfully submitted that the Examiner's objection has been overcome.

For the reasons set forth above, the Applicants believe that all of the pending rejections have been adequately addressed and that the present claims are in condition for allowance, which action is respectfully requested. The Examiner is invited and encouraged to contact the undersigned directly if such contact will expedite the prosecution of this application to issue.

A check in the amount of $60.00 as fee for a one month extension of time for a small entity is enclosed. This amount is believed to be correct. However, the Commissioner is authorized to charge any deficiencies, or credit any overpayment, to Deposit Account 50-0220.

Respectfully submitted,

Karen A. Magri
Registration No. 41,965

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1: Mol Cell Biol. 1993 Sep;13(9):5814-28.

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eps15, a novel tyrosine kinase substrate, exhibits transforming activity.

Fazioli F, Minichiello L, Matoskova B, Wong WT, Di Fiore PP.

Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892.

An expression cloning method which allows direct isolation of cDNAs encoding substrates for tyrosine kinases was applied to the study of the epidermal growth factor (EGF) receptor (EGFR) signaling pathway. A previously undescribed cDNA was isolated and designated eps15. The structural features of the predicted eps15 gene product allow its subdivision into three domains. Domain I contains signatures of a regulatory domain, including a candidate tyrosine phosphorylation site and EF-hand-type calciu binding domains. Domain II presents the characteristic heptad repeats of coiled-coil rod-like proteins, and domain III displays a repeated aspartic acid proline-phenylalanine motif similar to a consensus sequence of several methylases. Antibodies specific for the eps15 gene product recognize two proteins: a major species of 142 kDa and a minor component of 155 kDa, bo of which are phosphorylated on tyrosine following EGFR activation by EGF vivo. EGFR is also able to directly phosphorylate the eps15 product in vitro. addition, phosphorylation of the eps15 gene product in vivo is relatively receptor specific, since the erbB-2 kinase phosphorylates it very inefficiently Finally, overexpression of eps15 is sufficient to transform NIH 3T3 cells, th suggesting that the eps15 gene product is involved in the regulation of mitogenic signals.

PMID: 7689153 [PubMed - indexed for MEDLINE]

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1: J Cell Biol. 1997 Feb 24;136(4):811-21.

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Erratum in:
- J Cell Biol 1997 Apr 7;137(1):259.

Association and colocalization of Eps15 with adaptor protein-2 and clathrin.

van Delft S, Schumacher C, Hage W, Verkleij AJ, van Bergen en Henegouwen PM.

Department of Molecular Cell Biology, Institute of Biomembranes, Utrecht University, The Netherlands.

Eps15 has been identified as a substrate of the EGF receptor tyrosine kinase. In this report, we show that activation of the EGF receptor by either EGF or TGF-alpha results in phosphorylation of Eps15. Stimulation of cells with PDGF or insulin did not lead to Eps15 phosphorylation, suggesting that phosphorylation of Eps15 is a receptor-specific process. We demonstrate that Eps15 is constitutively associated with both alpha-adaptin and clathrin. Upon EGF stimulation, Eps15 and alpha-adaptin are recruited to the EGF receptor. Using a truncated EGF receptor mutant, we demonstrate that the regulatory domain of the cytoplasmic tail of the EGF receptor is essential for the binding of Eps15. Fractionation studies reveal that Eps15 is present in cell fractions enriched for plasma membrane and endosomal membranes. Immunofluorescence studies show that Eps15 colocalizes with adaptor protein-2 (AP-2) and partially with clathrin. No colocalization of Eps15 was observed with the early endosomal markers rab4 and rab5. These observations indicate that Eps15 is present in coated pits and coated vesicles of the clathrin-mediated endocytic pathway, but not in early endosomes. Neither AP-2 nor clathrin are required for the binding of Eps15 to coated pits or coated vesicles, since in membranes lacking AP-2 and clathrin, Eps15 still shows the same staining pattern. These findings suggest that Eps15 may play a critical role in the recruitment of active EGF receptors into coated pit regions before endocytosis of ligand-occupied EGF receptors.

PMID: 9049247 [PubMed - indexed for MEDLINE]

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1: Cancer Res. 1997 Dec 15;57(24):5498-504. Related Articles, Links

eps15 and eps15R are essential components of the endocytic pathway.

Carbone R, Fre S, Iannolo G, Belleudi F, Mancini P, Pelicci PG, Torrisi MR, Di Fiore PP.

Department of Experimental Oncology, European Institute of Oncology, Milan, Italy.

eps15 and eps15R are substrates of the epidermal growth factor (EGF) receptor kinase that are characterized by the presence of a protein:protein interaction domain, the EH domain, and by their ability to bind to the clathrin adaptor protein complex adaptor protein 2. Indirect evidence suggests that eps15 and eps15R are involved in endocytosis. Here we show that microinjection of antibodies against eps15 and eps15R inhibits internalization of EGF and transferrin. In addition, fragments of eps15 (encompassing its EH domains or the COOH-terminal region that binds to adaptor protein 2) inhibit EGF internalization or endocytosis of Sindbis virus. These results demonstrate that eps15 and eps15R are essential components of the endocytic machinery.

PMID: 9407958 [PubMed - indexed for MEDLINE]

1: FEBS Lett. 1998 Jan 30;422(2):129-31. Related Articles, Link

Heregulin beta1 induces the down regulation and the ubiquitin-proteasome degradation pathway of p185HER2 oncoprotein.

Magnifico A, Tagliabue E, Ardini E, Casalini P, Colnaghi MI, Menard S.

Division of Experimental Oncology E, Istituto Nazionale per lo Studio e la Cu dei Tumori, Milan, Italy.

Analysis of the fate of the p185HER2 oncoprotein following activation by heregulin beta1 revealed the induction of the tyrosine-phosphorylation, down-modulation, and polyubiquitination of p185HER2. Receptor ubiquitination was suppressed in cells treated with heregulin beta1 in the presence of sodium azide an inhibitor of ATP-dependent reactions, or genistein, a tyrosine kinase protein inhibitor, indicating the requirement for kinase activity and ATP in p185HER2 polyubiquitination. Ubiquitinated p185HER2 was degradated by the 26S proteasome proteolytic pathway. Kinetics and inhibition experiments indicated that endocytosis of the receptor occurs downstream of the initiation of the degradation process.

PMID: 9489990 [PubMed - indexed for MEDLINE]

1: Trends Cell Biol. 1998 Aug;8(8):299-301.

Related Articles, Link

Endocytosis proteins and cancer: a potential link?

Floyd S, De Camilli P.

Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510, USA.

Recent studies have shown that a variety of proteins participate with clathrin an clathrin adaptors in receptor-mediated endocytosis. The genes encoding some o these proteins are targets of chromosomal rearrangements in human haematopoietic malignancies. In addition, abnormal expression or mutation of some endocytosis proteins has been reported in human cancers. This article discusses these observations and elaborates the potential mechanisms by which the abnormal expression of endocytosis proteins might participate in the biolog of cancer.

Publication Types:

- Review
- Review, Tutorial

PMID: 9704404 [PubMed - indexed for MEDLINE]

Endocytosis is inhibited in hepatocytes from diabetic rats

Diabetes, Sept, 1993 by Joachim Krischer, Anne Gilbert, Phillip Gorden, Jean-Louis Carpentier

The consequences of type I diabetes on cellular endocytosis were investigated by comparing [I.sup.125]insulin, [I.sup.125] [alpha.sub.2]-macroglobulin, and Lucifer yellow uptake in hepatocytes freshly isolated from control and STZ-induced diabetic rats. In addition to the previously described reversible inhibition of ligand-induced internalization of the insulin receptor, we report a decrease in the constitutive receptor-modiated endocytosis of a [alpha.sub.2]-macroglobulin and a near abolition of fluid-phase endocytosis of Lucifer yellow in cells from diabetic animals. Despite decreased receptor autophosphorylation and internalization, the ligand-induced surface redistribution of the insulin receptor was normal in the diabetic cell population. By contrast, the insulin receptor association with clathrin-coated pits was impaired in diabetic cells as a result of a decreased concentration of these specialized invaginations on the nonvillous cell surface. The morphology and diameter of clathrin-costed pits were similar in both conditions under study. These results demonstrate a general impairment of endocytosis in hypoinsulinemic diabetes: receptor-medisted endocytosis was less affected than fluid-phase endocytosis. Impaired endocytosis of specific ligands or other macromolecules could be an important mechanism underlying the accumulation of extracellular matrix or even blood cholesterol removal in diabetes. Diabetes 42:1303-309,1993

Insulin-induced internalization of the insulin receptor is a multistep process involving surface and intracellular events (1). Ligand specificity is determined very early after the initial interaction between insulin and its receptor. The redistribution of insulin-receptor complexes from microvilli where binding occurs to the nonvillous region of the cell surface is ligand specific, because the redistribution depends on insulin-induced receptor kinase activation and autophosphorylation (2). By contrast, the second step is relatively nonspecific and common to many receptors, including those that are internalized even in the absence of their ligand. This step consists in the anchoring of the complex to the internalization gates, i.e., the clathrin-coated pits. This event does not require receptor kinase activation or autophosphorylation but instead requires the presence of Tyr containing [beta]-turns in the juxtamembrane domain of the receptor cytoplasmic tail (3-5).

Receptor-mediated endocytosis of [I.sub.125]insulin is impaired in hepatocytes of STZ-induced diabetic rats and monocytes of humans with...